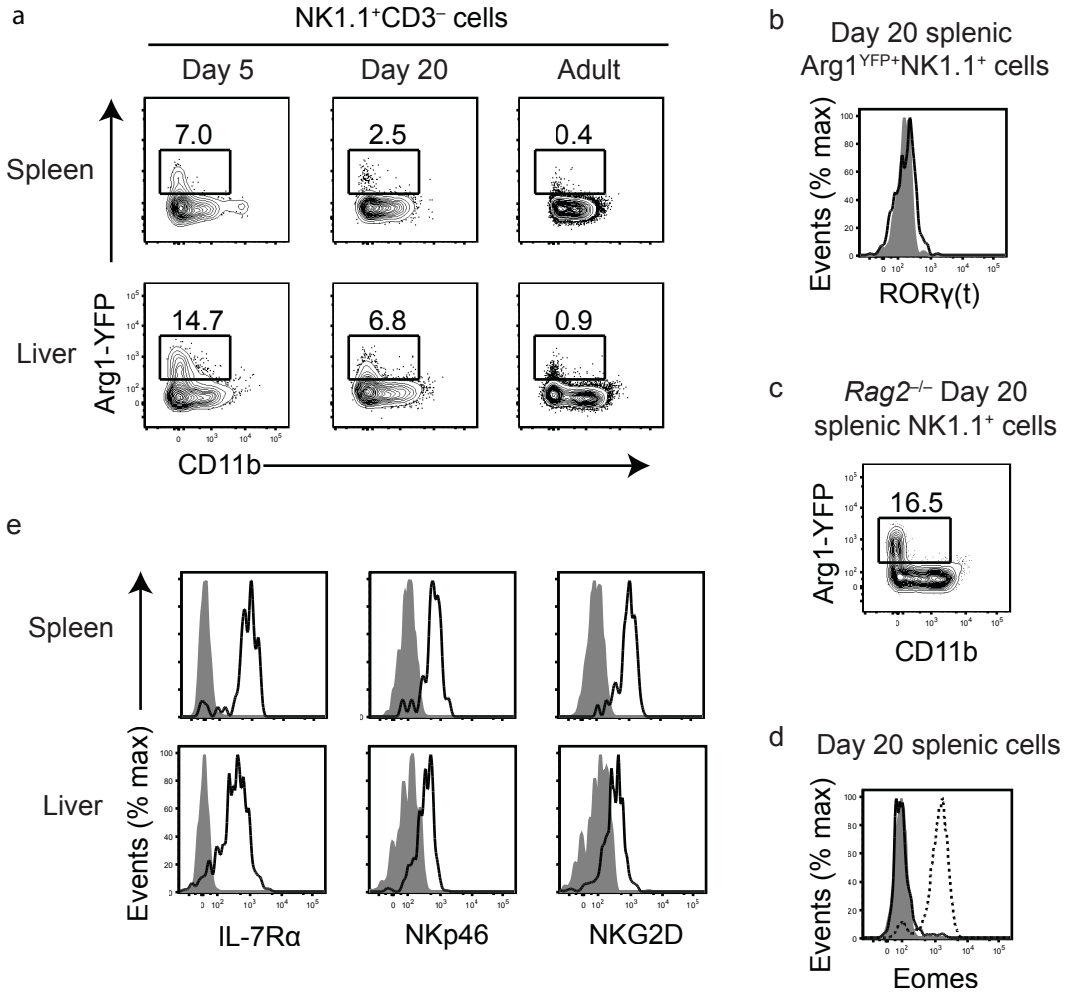
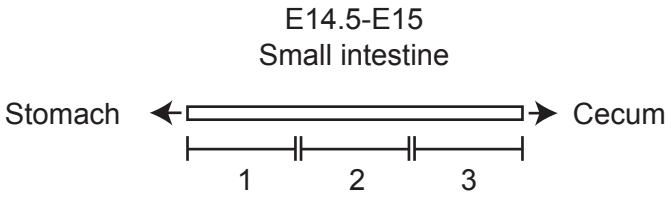
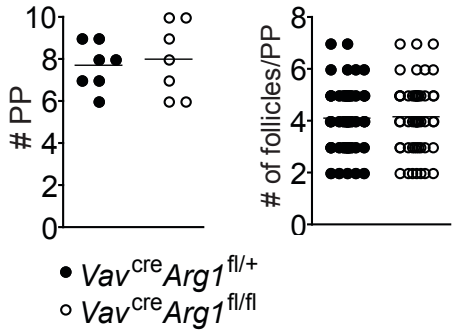


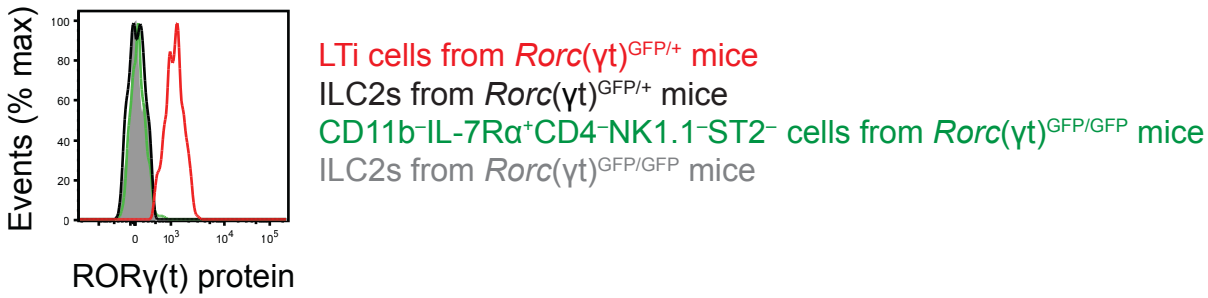
Supplementary Fig. 1. Characterization of Arg1^{YFP+}NK1.1^{+} cells. (**a**) YFP expression in NK1.1^{+}CD3^{-} cells from the spleen and liver of Arg1^{YFP} mice at different ages post-birth. (**b**) RORγ(t) protein expression in YFP^{+}NK1.1^{+} cells sorted from spleens of 20-day-old Arg1^{YFP} mice. The gray shaded area indicates CD4^{+} T cells. (**c**) YFP expression in NK1.1^{+} cells from *Arg1*^{YFP}*Rag2*$^{–/–}$ spleens of 20-day-old mice. (**d**) Eomesodermin expression in YFP^{+}NK1.1^{+} cells sorted from spleens of 20-day-old Arg1^{YFP} mice (solid line). The gray shaded area represents an isotype control and the dotted line represents sorted YFP$^{–}$NK1.1^{+}CD5$^{–}$ NK cells. (**e**) Expression of IL-7Rα, NKp46, and NKG2D in adult spleen and liver. Shaded areas indicate isotype controls. Data are representative of 2 independent experiements (**a-e**).



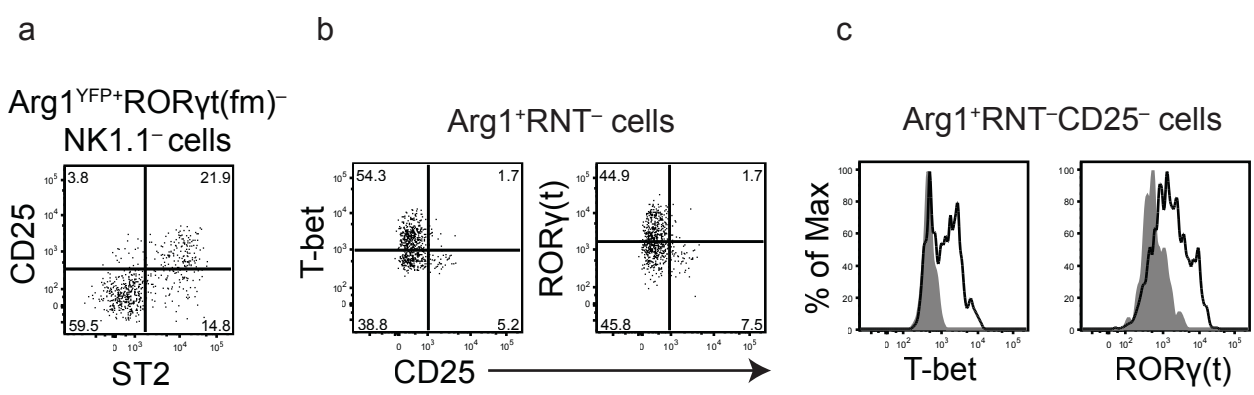
Supplementary Fig. 2. Division of the fetal small intestine into 3 regions of equal length for flow cytometry experiments in Fig. 3a.



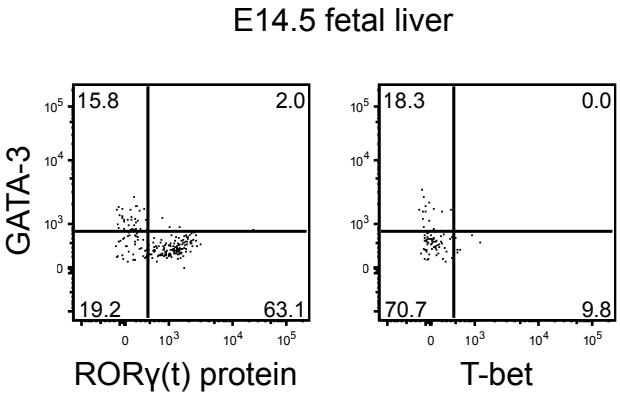
Supplementary Fig. 3. Arg1 is not required for PP development. PP numbers per intestine (left) and follicles per PP (right) in *Vav*^{cre}*Arg1*$^{fl/fl}$ mice and *Vav*^{cre}*Arg1*$^{fl/+}$ controls (n = 7 mice per group or n = 54-56 PP). P>0.05 (unpaired Student's t-test). Data are pooled from two independent experiments.



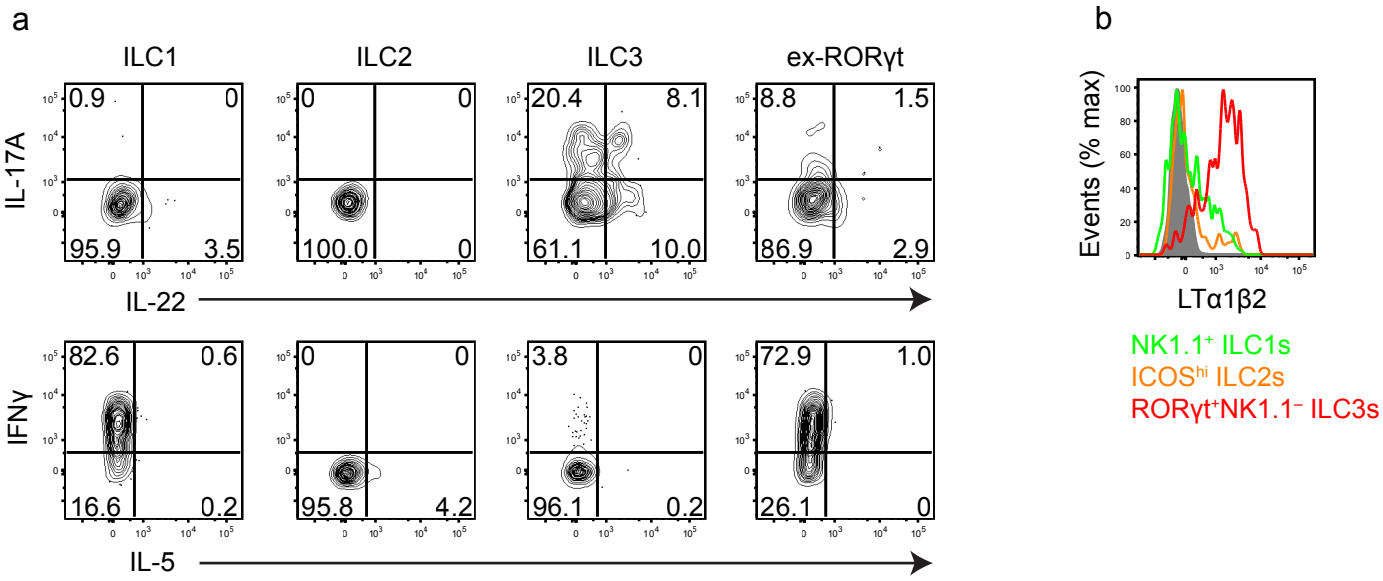
Supplementary Fig. 4. Specificity of RORγ(t) antibodies to RORγt in fetal Arg1$^+$ ILCs. *Rorc*(γt)$^{GFP/+}$ or *Rorc*(γt)$^{GFP/GFP}$ (knockout) cells from E16.5 intestines were stained with RORγ(t) antibodies. Cells were pre-gated on CD45$^+$CD11b$^-$IL-7Rα$^+$ cells, and then further gated on CD4$^+$NK1.1$^-$ cells for LTis, ST2$^+$NK1.1$^-$ cells for ILC2s, or CD4$^-$NK1.1$^-$ST2$^-$ cells to enrich for RNT$^-$ cells. Data are representative of two independent experiments.



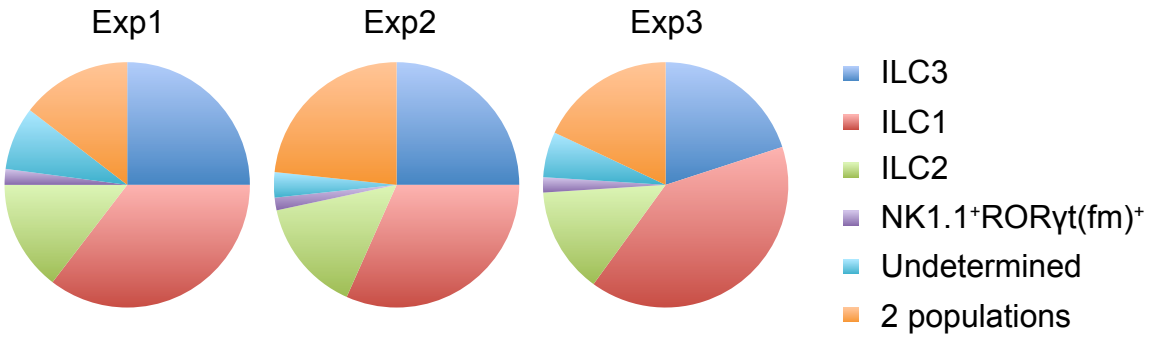
Supplementary Fig. 5. A subset of Arg1^{+}RNT^{-} cells expresses CD25. (a) CD25 staining in Arg1^{YFP+}RORγt(fm)$^{-}$NK1.1^{-} cells isolated from E15.5 fetal intestines. CD25 expressing RNT^{-} cells are shown in the top left quadrant. (b) T-bet and RORγ(t) expression in Arg1^{YFP+}RNT^{-}CD25^{-} (left quadrants) and Arg1^{YFP+}RNT^{-}CD25^{+} cells (right quadrants) isolated from E16.5 fetal intestines. (c) Histograms for T-bet and RORγ(t) expression in Arg1^{YFP+}RNT^{-}CD25^{-} cells. Data are representative of two independent experiments.



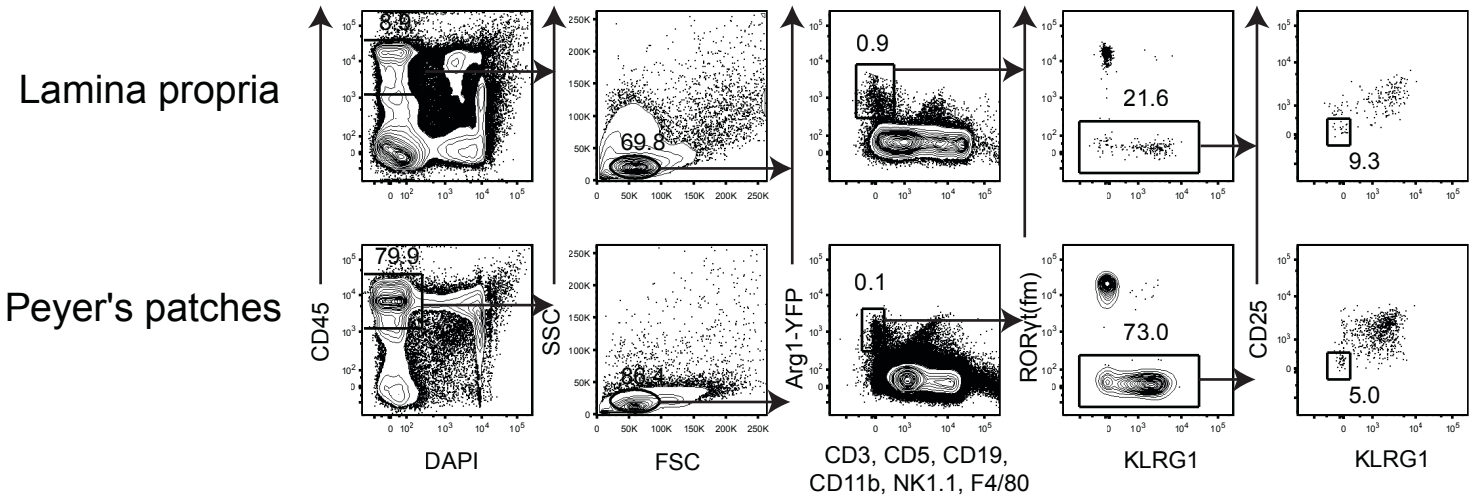
Supplementary Fig. 6. Transciption factor staining in E14.5 Lin$^-$IL-7Rα$^+$α$_4$β$_7^+$Flt3$^-$CD25$^-$ST2$^-$ fetal liver cells. Data are representative of three independent experiments.



Supplementary Fig. 7. Functional assessment of ILCs derived from Arg1^{YFP+}RNT^{-} cells. (**a**) Cytokine expression by 3 h PMA-and Ionomycin-stimulated ILCs at day 10 of culture. (**b**) LTα1β2 expression by ILCs at day 10 of culture. Data are representative of two independent experiments (**a-b**).



Supplementary Fig. 8. Variation between experiments pooled for Fig. 5H. Each pie represents an independent experiment in which 96 single Arg1^{YFP+}RNT^{-} cells were sorted and cultured for 6 days.



Supplementary Fig 9. Arg1^{+}RNT^{-} cells in adult intestinal tissue. (A) CD45^{+}, lymphocyte-sized Arg1^{+} cells in adult lamina propria and Peyer's patches include NK1.1^{-}RORγt(fm)$^{-}$KLRG1^{-}CD25^{-} cells. Data are representative of two independent experiments.